

Sequence determines degree of knottedness in a coarse-grained protein model

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Knots are abundant in globular homopolymers but rare in globular proteins. To shed new light on this long-standing conundrum, we study the influence of sequence on the formation of knots in proteins under native conditions within the framework of the hydrophobic-polar (HP) lattice protein model. By employing large scale Wang-Landau simulations combined with suitable Monte Carlo trial moves we show that, even though knots are still abundant on average, sequence introduces large variability in the degree of self-entanglements. Moreover, we are able to design sequences which are either almost always or almost never knotted. Our findings serve as proof of concept that the introduction of just one additional degree of freedom per monomer (in our case sequence) facilitates evolution towards a protein universe in which knots are rare.

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Knots have fascinated physicists, mathematicians and chemists for a long time. About 140 years ago, Kelvin hypothesized that atoms consist of knots in the aether [1]. At first sight, this beautiful idea is quite appealing as knots are, in a sense, unique and just like atoms cannot change their type: Without breaking bonds a simple unknotted ring (a so-called unknot) cannot be, e.g., transformed into a trefoil knot (3_1 , with three minimal crossings in a projection onto a plane). But as this aesthetically pleasing model was finally rejected most of the initial enthusiasm among natural scientists faded, and knot theory became truly a part of mathematical sciences. In recent decades, however, the field went through a renaissance spurred by the discovery of knots in DNA [2, 3] and proteins [4–6].

Knotted proteins in particular pose a number of challenges which are not overcome easily and question our understanding of evolution and folding - especially when we keep in mind that the function of a protein is determined by its three-dimensional structure. Only eleven folds are known to be knotted (one of which has been created artificially) and most of these knots are simple trefoils [6]. There is also one protein knot with five crossings which incidentally makes up 1-2 % of our brain protein mass, (pdb-code:2etl) [7], and there is even a knot with six crossings (pdb-code:3bjx) [8]. Indeed, it is difficult to imagine how such proteins always fold into their knotted native state [9]. A number of experiments have shown that certain knotted proteins can refold to the knotted state upon degradation [10] and that the process can be accelerated by chaperons [11]. From a topological point of view folding may not always be as difficult as it appears in the first place though, as even complicated knots (e.g., the 6_1 knot mentioned above) can be generated from an unknotted state by a single global movement of a subchain as shown by coarse-grained folding simulations with Gō-models [8].

The apparent rarity of knotted proteins is in stark contrast to the abundance of knots in globular polymers [12–

15]. Even though proteins are not archetypal homopolymers of the bead-spring type, this discrepancy is nevertheless remarkable. Indeed, there are several competing (and even complementing) ideas why knots are rare. Taylor and Lin [16] pointed out that proteins should rather be compared to a chain of “sticky beads” – a visualization of an old idea [17]: The protein essentially folds from an unknotted swollen state and remains in an unknotted (“crumbled”) globular state which results from the initial collapse. From a structural point of view the emergence of secondary structure also changes the length-scale at which knots occur and likely decreases their probability of occurrence. A first systematic study in this context was undertaken by Lua and Grosberg [18]; they compared the scaling of subchains between real proteins and compact lattice loops and found that, statistically, proteins tend to “fold back on themselves” at intermediate scales up to 40 amino acids which may act as a strong suppressor of knotting. To which extent this is a result of evolution working towards the suppression of knots (as they may be adverse to folding or function) is still largely unknown.

In this letter we focus on how such mechanisms may have evolved in the first place. Consider a statistical ensemble of (potentially highly knotted) globular proteins made up of random amino acids with a certain degree of variability. Natural selection has led to a “Protein Universe” [19, 20] significantly different from the statistical average of our random amino acid chains - apparently full of purpose and function and with little or no knots.

Within the framework of a minimalist protein model, the hydrophobic-polar (HP) lattice model [21–23], we show that a single additional degree of freedom per monomer, namely sequence, may provide an evolutionary pathway which allows proteins to evolve towards a “lattice protein universe” which is almost void of knots. We are able to design sequences and identify patterns, which suppress or enhance the formation of knots in our lattice model. However, due to the coarse-grained nature

of the lattice, these sequences are typically not the same as in real proteins which are considerably more complex.

In the HP model the protein is represented as a self-avoiding chain of beads (the amino acid residues) on a regular lattice (here, simple cubic). There are only two classes of amino acids, hydrophobic (H) and polar (P) residues. Proteins as opposed to homopolymers have a hydrophobic core resulting from the tendency of shielding the hydrophobic side-chains from the polar (aqueous) environment. In the HP model this hydrophobic force is (implicitly) mimicked by an attractive interaction ϵ that acts between non-bonded neighboring H residues ($\epsilon_{HH} = -1$, $\epsilon_{HP,PP} = 0$). Thus, at low temperatures H residues tend to gather in the interior of the globular state and form a hydrophobic core while P residues are located at the outer shell. Despite its limitations [24, 25], the HP model has been widely used to describe protein folding qualitatively and to shed new light onto some of the most puzzling questions in protein science (e.g., Levinthal and blind watchmaker paradox [20], chaperonin-mediated protein folding [26], mutation-induced fold switching [27], to mention a few). Thus, it also serves as a good starting point to address the questions of knottedness (a fundamental, topological property of proteins) at the level of abstraction of the present study. While the result of a successful folding process along a folding funnel is generally assumed to correspond to a free energy minimum [20, 28], our simulations generate conformations in the close vicinity of this minimum, which are subsequently analyzed with respect to knots [29].

In order to address the problem from a statistical point of view we need to sample a large ensemble of random protein sequences under *native* conditions (i.e., ground-state like). To make sure that lattice effects do not bias the statistics, long chains lengths ($N > 100$) are required [30]. Together, these requirements pose a considerable challenge on the computational procedure and, thus, a similar systematic study has not been carried out for any type of protein model so far. Even for the very simplified HP model, estimating the ground-state of a specific HP sequence has only been possible up to around 100 monomers with state of the art techniques and computational power [31–33].

Recently, however, Wüst and Landau [34] proposed an efficient Monte Carlo scheme which renders the sampling of *uncorrelated*, low-energy (i.e., “native like”) structures feasible even for chain lengths up to $N = 500$. The key of their procedure is the combination of Wang-Landau (WL) sampling [35] with two non-traditional Monte Carlo trial moves, namely pull moves [36] and bond-rebridging moves [37] which complement each other extremely well. Their methodology has proven to be very powerful in overcoming both the energetic and entropic barriers typically encountered when sampling the complex free energy landscape of dense lattice polymers and

proteins. For details, see [34].

For the topological characterization of protein conformations we need to compute so called knot invariants which are only unique for closed curves (mathematically, knots are only well-defined for closed curves). Thus, for linear polymers and proteins the notion of knottedness needs to be extended to open chains by choosing a particular closure which connects the termini in a well-defined manner (thus closing the loop) [38–40]. It is important that the closure itself has no significant influence on the calculation of the knot invariants. Even though some ambiguity remains, different closures typically yield similar results from a statistical point of view [7, 14]. In this paper we use a rather simple closure which was already applied successfully for the determination of knots in real proteins: We determine the center of mass of the polymer and draw two lines through the first and the last bead. Outside the protein the two lines are connected by a straight line. From this structure we compute the Alexander polynomial (knot invariant). The numerical implementation of the entire procedure is described in great detail in [7, 41].

Fig. 1 shows the unknotting probabilities for 100 random HP sequences and a few designed HP sequences under native conditions. All chains consist of $N = 500$ monomers with 50 % H and 50 % P residues (except for the homopolymer with 100 % H). This chain length was chosen such that the homopolymer already exhibit a significant amount of knotting. We have also studied shorter chains (down to $N = 100$) and obtained qualitatively similar results even though the overall probability to find a knot for shorter chain lengths is, of course, correspondingly smaller. In each simulation the sequence of a chain is fixed and does not vary. Thus, we investigate an ensemble of sequences to show how the introduction of this additional degree of freedom per monomer may affect an evolutionary system.

It is worth noting that the HP model exhibits a rather large ground-state degeneracy, which could be reduced somewhat by adding additional interactions between H and P monomers. The degeneracy of the ground-state and the additional states in its vicinity allowed us, however, to determine a “likelihood” of knottedness for a given HP sequence as follows: First, a pre-WL run was performed to obtain an estimate of its ground-state energy. Then, a subsequent production WL simulation, restricted to the lowest 20 % of the entire energy range, consecutively sampled conformations within 5 % of the ground-state energy. (This threshold was set heuristically but other values < 10 % gave similar results). Between the sampling of any two conformations the random walker must always perform a full round trip through the specified energy range in order to reduce possible structural correlations. Multiple, independent production WL simulations were run simultaneously to speed up the sampling and further increase the structural diversity

segments in the designed sequence #1, which is almost always unknotted, induces a very regular, slab-shaped, native structure with H monomers filling the interior of the slab and P monomers occupying its border. This compact structure results from a distinct local threading of the sequence which disfavors entanglements. Alternating sequences of H_4P_4 and H_2P_2 segments have a similar effect (cf. snapshot in Fig. 1), but structures tend to be ellipsoidal rather than flat. Another, almost trivial motive are simple alternating H and P monomers, which form a swollen coil structure akin to a self-avoiding walk (cf. snapshot in Fig. 1). In contrast, a pattern which highly favors the formation of knots is presented in the designed sequence #2. It consists of long contiguous segments of H and P residues separated by segments of repeating (HP) motives. This sequence forces the protein to fold back through extended loops in order to optimize the number of non-bonded HH interactions and there is almost no local order inside the hydrophobic core. Both features foster entanglements and knots. Again, we need to stress that there is no one-to-one correspondence between motives which enhance or suppress knots in lattice proteins and real proteins, which are considerably more complex. Indeed, for the latter such motives have not even been identified.

Despite the variation in the degree of knottedness, all native like structures of HP sequences exhibit a more or less pronounced hydrophobic core. Thus, the formation of a hydrophobic core in itself cannot be considered as a precursor of suppression of knots in proteins. However, the local structure (order) among residues within a sequence strongly influences knotting as manifested by the index coloring scheme of corresponding structures (see lower row of snapshots in Fig. 2). Whereas in the designed sequence #1 nearby monomers are strongly localized and form a precursor of secondary structure, in the designed sequence #2 they tend to spread out far and in uncorrelated directions. In real proteins individual elements of secondary structure have the tendency to fold back onto themselves which, in turn, introduces locality and suppresses knots [18]. It is remarkable that the HP sequences studied here show the same relationship between knottedness and local structure despite the simplicity of the underlying protein model.

Finally, we compare the average knotting probability of random hetero- and homopolymers as a function of solvent quality (i.e., temperature in our model) ranging from ground-state like structures, in which knots tend to spread over the whole structure, to the denatured case, in which they are weakly localized (not shown here). To make a fair comparison, we plot the probability of observing an unknotted structure (or a trefoil knot) as a function of the radius of gyration. To be able to define an average knotting probability for random heteropolymers, we have again averaged over our 100 random HP sequences; Metropolis Monte Carlo sampling (using the

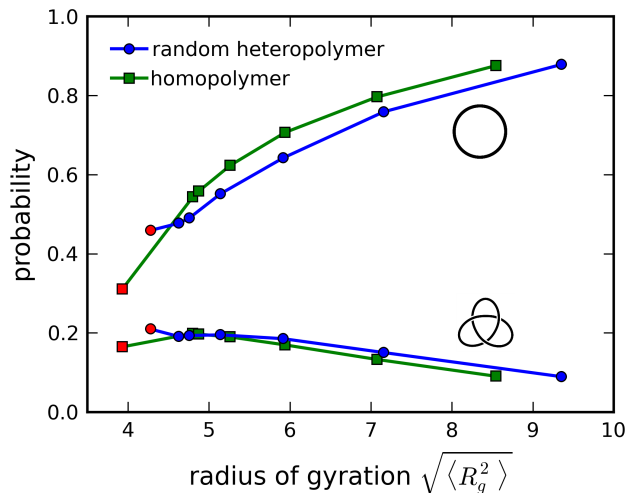


FIG. 3. Average probabilities of finding unknots (*upper curves*) and trefoils (*lower curves*), respectively, in homopolymers (100 % H) and random heteropolymers (50 % H, 50 % P) with $N = 500$ as a function of the root mean squared radius of gyration, $\sqrt{\langle R_g^2 \rangle}$. The red symbols denote corresponding probabilities under native condition (ground-state like). Error bars have been calculated by averaging over independent runs; they do not exceed symbol size and are, thus, not shown.

same move sets as described above) has been employed to obtain correctly weighted estimates at finite temperatures. Fig. 3 shows that the probability of finding unknots or trefoil knots in heteropolymers (averaged over random sequences) is quite similar to the one for homopolymers at comparable densities. However, at high densities (low temperatures) the unknotting probability of heteropolymers clearly deviates from the decreasing trend observed in homopolymers.

In this study we have been able to demonstrate quantitatively that sequence strongly influences (or even determines) the degree of knottedness under native conditions. Within the framework of the minimalist HP protein model and large scale Monte Carlo simulations, we have determined probabilities of knotting for random HP sequences as well as homopolymers with 500 residues. The introduction of sequence leads to a large variability in the self-entanglements of heteropolymers even though on average they are almost as knotted as globular homopolymers of comparable density. We have also been able to design sequences which fold into either highly knotted or almost knot-less structures. While we demonstrate that a variation of sequence leads to a variation of self-entanglements and knots it is likely that variability in other interactions may have similar effects. This shows in principle that the introduction of a single additional degree of freedom per monomer, in our case sequence, may already suffice to facilitate evolution towards a largely unknotted “Protein Universe”. In a sense, proteins are

not an equilibrium ensemble of (knotted) random heteropolymers and should as such not be compared to an equivalent ensemble of homopolymers, but instead live in a very specific conformational subspace in which knots are rare.

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